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HbA1c levels in iron deficiency anemia cases grouped according to hemoglobin levels

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Abstract

Aim: Iron deficiency anemia (IDA) is a prevalent nutritional deficiency anemia affecting global populations, characterized by impaired hemoglobin synthesis and erythrocyte dysfunction. Hemoglobin A1c (HbA1c) levels, crucial for monitoring glycemic control in Diabetes Mellitus (DM), may be influenced by factors beyond blood glucose, including anemia. This study aims to evaluate the impact of IDA on HbA1c levels in non-diabetic individuals aged 15-60.

Materials and Methods: A retrospective cross-sectional study conducted. The study included 299 IDA patients with FBG and HbA1c levels within the reference range (70-100 mg/dL for FBG, 4%-6.0% for HbA1c) and 114 healthy controls. Exclusion criteria encompassed DM history, recent iron therapy, other anemias, chronic infections, and malnutrition. Patients were grouped by anemia severity regarding hemoglobin levels: mild (11-11.9 g/dL), moderate (10.1-10.9 g/dL), and severe (<10 g/dL). Statistical analysis involved ANOVA, Tukey test, chi-square test, and Pearson correlation, with significance set at $p<0.05$.

Results: Mean HbA1c levels were 5.42±0.34%, 5.48±0.27%, 5.42±0.33%, and 5.28±0.27% in mild, moderate, severe IDA, and control groups, respectively, showing no significant difference among IDA groups but significantly lower in controls ($p<0.001$). A low negative correlation existed between HbA1c and hemoglobin, iron, and ferritin levels ($p<0.01$ for Hb and Fe, $p<0.05$ for ferritin). Age correlated moderately positively with HbA1c ($p<0.01$).

Conclusion: The mechanism of the relationship between iron deficiency anemia and HbA1c levels has not been fully explained. This study highlights the significant influence of IDA on HbA1c levels in non-diabetic individuals aged 15-60, emphasizing the need for larger, prospective studies to clarify this relationship and enhance the interpretation of HbA1c.

Introduction

The fact that anemia, a disease characterized by a hemoglobin level below the lower normal range, affects a significant part of the world's population is concerning [1]. About one-third of elderly people suffer from a nutritional deficiency—such as low iron, folate, or vitamin B12—that results in anemia. Iron deficiency is the most frequent dietary cause of nutritional deficiency anemia, and iron is essential for hemoglobin's ability to carry oxygen. Iron is also necessary for the many enzymes to function properly, as well as for the transport of oxygen, the synthesis of DNA, RNA, and proteins, and the production of energy [2].

One prevalent worldwide health issue is iron deficiency anemia (IDA). Reduced iron absorption, high iron loss, and insufficient oral intake are the three most common causes of IDA [3]. Increased total iron-binding capacity (TIBC), reduced serum iron and ferritin levels, hypochromia and microcytosis in erythrocytes, and transferrin saturation below 15% are the hallmarks of IDA. Ferritin levels <15 µg/L are the most commonly used criterion for diagnosing IDA [4]. Ferritin levels can also rise as a result of inflammatory or infectious conditions.

For patients with Diabetes Mellitus (DM), maintaining glycemic control is essential to reducing the risk of complications. Glycated hemoglobin (HbA1c), fructosamine, and fasting blood glucose (FBG) are the metrics used to track glycemic control. Because it represents glycemic control over the previous six to eight weeks and is fre-

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quently used in routine practice to monitor glycemic control, HbA1c is a noteworthy parameter [5]. HbA1c levels are influenced by blood glucose levels and conditions affecting erythrocyte survival, such as variant hemoglobin presence, hemolytic anemias, nutritional anemias, uremia, pregnancy, and acute blood loss [6].

Studies have demonstrated a relationship between IDA and HbA1c levels. Eventhough the precise mechanism underlying this relationship is unclear, possible reasons deserve attention. Elevated HbA1c levels can result from a higher proportion of older erythrocytes in IDA cases [7]. Furthermore, elevated oxidative stress in IDA may cause inflammatory molecules to be released, which would improve hemoglobin glycation [8]. Moreover, the relationship between IDA and HbA1c levels may be explained by the relative increase in glycated hemoglobin brought on by a decrease in total hemoglobin concentration [9]. In addition, chronic disease-related anemia can also have elevated HbA1c levels.

DM and IDA are two common conditions in the population. Numerous studies have demonstrated that IDA affects HbA1c levels, which are both a diagnostic criterion for DM and an indicator of long-term glucose regulation. This study aims to evaluate the impact of IDA on HbA1c levels in a cohort of non-diabetic individuals aged 15-60.

Materials and Methods

Our study strictly adhered to the principles outlined in the Declaration of Helsinki. Ethical approval for the study was obtained from the Ethics Committee of Ankara Dr Sami Ulus Training and Research Hospital on 01.06.2022, protocol number E-2022/06-350. Informed consent was not obtained from the patients as the study involved a retrospective analysis of anonymized blood results used solely for disease monitoring.

Patient selection

This study is a retrospective and cross-sectional study. The study included 299 IDA patients with FBG and HbA1c levels within the reference range (70-100 mg/dL for FBG, 4%-6.0% for HbA1c) and 114 healthy controls. Exclusion criteria for the study were as follows: known history of DM, use of drugs such as corticosteroids that cause insulin resistance, use of iron therapy in the last three months, presence of continuous medication, history of malignancy, history of anemia due to other causes (hemolytic anemia, thalassemia, etc.), history of recent acute or chronic infection, Cushing's syndrome, liver and kidney disease, malignancy, malnutrition and malabsorption, and presence of any disease other than iron deficiency anemia. The clinical information of the participants was obtained from our hospital's Clinical Information Operating System and laboratory information was obtained from the Laboratory Information Operating System.

Grouping and cut-off values

A cut-off value of Hb <12 g/dL was used to define anemia for both sexes. Iron <37 µg/dL and ferritin <15 µg/L were used as cut-off values for IDA. The patient group was divided into mild IDA group (11-11.9 g/dL), moderate IDA group (10.1-10.9 g/dL), and severe IDA group (<10 g/dL) according to Hb levels. For the control group, men with Hb levels between 13-17 g/dL and iron levels within the reference range of 59-158 µg/dL and women with Hb levels between 12-15 g/dL and iron levels within the reference range of 37-145 µg/dL were selected and included in the study. For all tests, reference range values are those recommended by the manufacturer.

Laboratory measurements

HbA1C levels were determined by the ion exchange HPLC method on a Lifotronic H9 device. Complete blood count parameters were analyzed by a Sysmex XN-1000 device (Roche DiagnosticsGmbH, Mannheim, Germany). Glucose levels were analyzed using the hexokinase method and unsaturated iron binding capacity and iron levels were analyzed using the FerroZine method on the AU 5800 device (Beckman Coulter). Ferritin levels were analyzed using the chemiluminescence method using Advia Centaur XPT (Siemens Healthcare).

Statistical analysis

The normal distribution of the variables was tested by Kolmogorov Smirnov test, and the equality of variance was tested using Levene's test. ANOVA test was used to determine whether the groups had a statistically significant difference. Tukey test was used to determine differences between groups. Chi-square test was performed to compare the gender parameter between the groups. The Pearson correlation test was used to investigate whether there was a correlation between the parameters. Descriptive statistics were shown as mean ± standard deviation. $P<0.05$ was considered statistically significant for all tests. All statistical analyses were conducted with IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp.). A priori power analyses were conducted using G*Power (version 3.1.9.7) to estimate the required sample sizes for the study. For the ANOVA test, with a significance criterion of $\alpha = 0.05$, an effect size $f = 0.25$, and power = 0.95, the minimum sample size required to compare 4 groups was 280. The actual sample size of the study was 413, which exceeds this minimum requirement, ensuring adequate power for the analysis. For the Pearson correlation, with a significance criterion of $\alpha = 0.05$, an effect size $r = 0.30$, and power = 0.95, the minimum sample size needed to detect correlation for each parameter was 292. The actual sample size of the study was 413, which is sufficient to achieve the desired power for detecting correlations.

Results

The data of the parameters in groups are shown in Table 1. The mean age of the patients included in our study was 36.10±10.83 years in the severe IDA group, 39.10±9.16 years in the moderate IDA group, 33.50±9.61 years in the mild IDA group and 33.60±11.55 years in the control group. There was no statistically significant difference between the ages of the mild and severe IDA groups and the control group ($p>0.05$). However, a significant age difference was found between the moderate IDA and control groups ($p<0.001$).

Table 1. Demographic and laboratory characteristics of the groups.

Parameters	Mild IDA (n:103)	Moderate IDA (n:98)	Severe IDA (n:98)	Controls (n:114)	p-value
Gender (Female/Male)	101/2	95/3	96/2	109/5	>0.05
Age (years)	33.50±9.61	39.10±9.16***	36.10±10.83	33.60±11.55	**<0.001**
Hb (g/dL)	11.48±0.34***	10.48±0.27***	8.94±0.96***	14.28±1.06	**<0.001**
Fe (µg/dL)	37.05±16.07***	28.12±11.77***	19.38±9.67***	85.60±35.07	**<0.001**
UIBC	391.60±55.5***	390.8±61.90***	422.50±50.8***	269.95±49.15	**<0.001**
Ferritin (ng/mL)	6.00±3.81***	4.65±3.20***	3.89±2.57***	36.19±32.98	**<0.001**
Glucose (mg/dL)	89.30±6.77	89.33±6.64	88.80±6.19	88.84±6.97	>0.05
HbA1c (%)	5.42±0.34**	5.48±0.27***	5.42±0.33**	5.28±0.27	**<0.001**

ANOVA test was used to determine whether the groups had a statistically significant difference. Tukey test was used to determine differences between groups. Mean and standard deviation values of the parameters according to the groups are shown. P -values less than 0.05 are considered statistically significant and are shown in bold. ** indicates p<0.01 and *** indicates p<0.001 according to Tukey test. Abbrevations: Hb, hemoglobin; Fe, iron; UIBC, unsaturated iron-binding capacity; IDA, iron deficiency anemia.

Table 2. Correlation analysis of parameters and HbA1c.

Parameters	r-value	p-value
Age (years)	0.402	**<0.01**
Hb (g/dL)	-0.228	**<0.01**
Fe (µg/dL)	-0.226	**<0.01**
UIBC	0.182	**<0.01**
Ferritin (ng/mL)	-0.100	**<0.05**
Glucose (mg/dL)	0.206	**<0.01**

Pearson correlation analysis between parameters and HbA1c. Hb, Fe, and ferritin showed a low negative correlation with HbA1c (p<0.01 for Hb and Fe, p<0.05 for ferritin). Age showed a moderate and glucose and UIBC showed a low positive correlation with HbA1c (p<0.01). Abbrevations: r, Pearson correlation coefficient; Hb, hemoglobin; Fe, iron; UIBC, unsaturated iron-binding capacity; IDA, iron deficiency anemia.

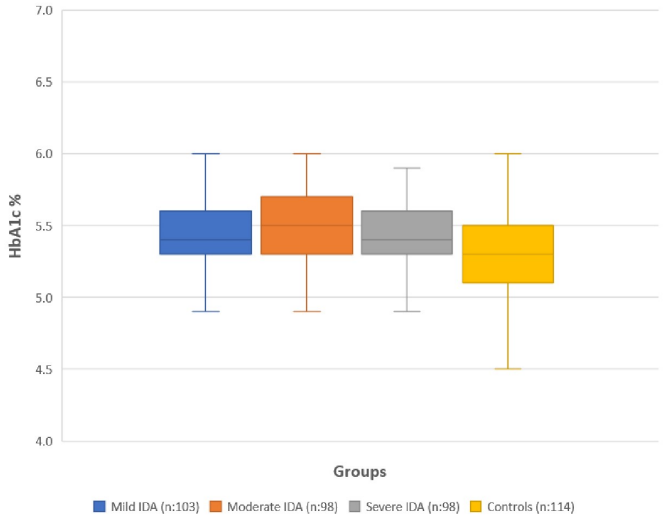

Figure 1. Box plot showing the distribution HbA1c values according to IDA groups.

The hemoglobin level of the mild IDA group was 11.48±0.34 gr/dL, the hemoglobin level of the moderate IDA group was 10.48±0.27 gr/dL, the hemoglobin level of the severe IDA group was 8.94±0.96 gr/dL, and the hemoglobin level of the control group was 14.28±1.06 gr/dL. The serum iron level in the mild IDA group was 37.05±16.07 µg/dL; in the moderate IDA group, it was 28.12±11.77 µg/dL; in the severe IDA group, it was 19.38±9.67 µg/dL and in the control group was 85.6±35.07 µg/dL. Tukey test showed that Hb and Fe parameters was significantly different among all groups (p<0.001).

HbA1c levels were 5.42±0.34% in mild IDA group, 5.48±0.27% in moderate IDA group, 5.42±0.33% in severe IDA group and 5.28±0.27% in control group, and no significant difference was found between the groups (p>0.05). We found that HbA1c values were significantly lower in the control group than in the IDA groups (p<0.001) (Figure 1).

Pearson correlation analysis indicated that Hb, Fe, and ferritin showed a low level negative correlation with HbA1c (p<0.01 for Hb and Fe, p<0.05 for ferritin) (Table 2). Age showed a moderate positive correlation with HbA1c (p<0.01), indicating that older individuals might exhibit higher HbA1c levels independent of glucose control. Additionally, glucose and UIBC showed a low level positive correlation with HbA1c (p<0.01).

Discussion

HbA1c, utilized in the diagnosis and treatment follow-up of diabetic patients, is not merely influenced by glucose levels but also by the presence of variant hemoglobin, hemolytic anemias, nutritional anemias, uremia, pregnancy, and acute blood loss. Iron deficiency anemia is one of the most prevalent nutritional anemias and is postulated to affect HbA1c levels. Our study evaluated HbA1c levels in different IDA groups and control groups and investigated the correlation between HbA1c levels and iron-related tests. Our study found no significant difference between groups in HbA1c levels in IDA cases grouped as mild, moderate and severe, but there was a significant difference in the control group compared with IDA groups. We also found a significant negative correlation between serum iron levels and HbA1c levels.

Several mechanisms can explain an increase in HbA1c levels among IDA patients. Iron deficiency leads to a prolonged lifespan of erythrocytes due to less available iron

for effective erythropoiesis. The long lifespan enhances hemoglobin exposure to glucose, which brings a rise in HbA1c levels. Iron deficiency also directly influences the process of glycation: the lack of adequate iron suppresses the work of enzymes that are responsible for the formation of hemoglobin, among which are ferrochelatase and aminolevulinic acid synthase. Eventually, such an influence amplifies the process of non-enzymatic formation of glycated hemoglobin, and thus increases HbA1c levels [10]. Oxidative stress is also increased during IDA, leading to changes in the red blood cell membrane along with variation in erythrocyte turnover. Oxidative damage produces a higher percentage of older more glycated red blood cells in circulation [11]. Hemoglobin structural variants causing iron deficiency can also interfere with common HbA1c assay methods yielding falsely elevated values.

In the review published by English et al. in 2015, they detected that anemia and anomalies in erythrocyte indices affect HbA1c levels in adults without diabetes, and it was stated that HbA1c is affected by iron deficiency and iron deficiency anemia and false elevation is observed [12]. Kim et al. determined significantly higher HbA1c levels in women with iron deficiency than those without, independent of fasting glucose levels, in a study of 10,535 individuals. The same study found no significant difference in HbA1c levels >6% [13]. A study by Koga et al. conducted on 104 participants found an association between iron deficiency (IDA) and increased HbA1c levels and showed a significant negative correlation between them [14]. Ford et al. conducted a study on 8296 participants; they indicated a borderline elevation in HbA1c in individuals with normal hemoglobin and low iron index compared to individuals with normal Hb and average iron index. This study concluded that in patients with low or high Hb levels and HbA1c between 5.7-6.5%, changes in erythrocyte turnover should be considered before diagnosing prediabetes, but this does not require screening for iron deficiency to determine the reliability of HbA1c in the diagnosis of prediabetes or diabetes [15].

The study by Coban et al. showed that the initially high HbA1c levels decreased significantly following iron treatment given to patients with iron deficiency anemia. It was stated that the high HbA1c levels in IDA could be explained by increased glycolyzed fraction due to a decrease in hemoglobin concentration when serum glucose was constant [16]. In the study published by Oğuz et al. in 2014 and conducted in normoglycemic individuals, mean HbA1c values were found to be 5.61% and 5.56% for IDA patient and control groups, and no significant correlation was found between HbA1c and hemoglobin, iron, and unsaturated iron-binding capacity levels [17].

Different test principles are used for HbA1c measurement. The most preferred are ion exchange chromatography, electrophoresis, affinity chromatography, and immunoassay techniques. Different results in the studies examining the effect of iron deficiency on HbA1c levels may be due to the difference in the characteristics and number of the patient groups evaluated and the difference in the HbA1c measurement method. Furthermore, the cross-sectional nature of our study represents a limitation. A prospective study design would have allowed for more effective results to be obtained by following the patients over time. Additionally, the lack of data on the duration of IDA diagnosis represents a further limitation. Because of the clinical course and oxidative stress or erythrocyte turnover changes become established with prolonged IDA.

Conclusion

In conclusion, the analysis of the effect of IDA on HbA1c remains inconclusive, as existing studies show different results. The mechanism of the association between IDA and HbA1c levels is also not fully explained. We concluded that studies evaluating the relationship between IDA and HbA1c levels should be conducted in larger patient groups and evaluated on different HbA1c systems.

Ethical approval

Ethical approval for the study was obtained from the Ethics Committee of Ankara Dr Sami Ulus Training and Research Hospital on 01.06.2022, protocol number E-2022/06-350.

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